Expert Opinion

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Na⁺/H⁺ exchanger-1: a link with atherogenesis?

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Importance of the field: The sodium/hydrogen exchanger-1 (NHE-1/SLC9A1) is a ubiquitous plasma membrane protein whose main role is maintenance of intracellular pH and volume. NHE-1 plays a role in atherogenesis; however, its clinical relevance has not yet been established.

Areas covered in this review: We herein review the contribution of NHE-1 in atherogenesis (namely its effect on endothelial cells, monocytes, smooth muscle cells and platelets).

What the reader will gain: Studies have shown that NHE is involved in atherogenesis-related properties of isolated monocytes. We also consider the relationship between NHE-1 and vascular risk factors such as obesity, diabetes mellitus, hypertension, dyslipidemia and inflammation.

Take home message: Even though clinical trials with certain NHE-1 inhibitors have had discouraging results, NHE-1 cannot be excluded as a potential future therapeutic target for the prevention and/or treatment of atherosclerosis.

Keywords: atherosclerosis, cariporide, endothelial cells, monocytes, platelets, smooth muscle cells, sodium-hydrogen antiporter

1. Introduction

The sodium/hydrogen exchanger-1 (NHE-1/SLC9A1) is a ubiquitous integral membrane protein expressed in mammalian cells [1]. Its main role is intracellular pH maintenance, which is achieved by exchanging 1 intracellular H^+ for 1 extracellular Na^+ [1,2]. It is also important for cell volume maintenance and cytoskeletal reorganization. Furthermore, it takes part in cell proliferation, apoptosis and migration [1]. NHE-1 activity is stimulated by intracellular acidosis, hormones and growth factors and inhibited by amiloride derivatives [3].

NHE-1 plays a role in atherogenesis; however, its clinical relevance has not yet been established. Atherogenesis is an inflammatory process starting at birth, which progresses continuously as age increases. However, the presence of risk factors such as obesity, diabetes, dyslipidemia, hypertension and smoking accelerates this process. Atherogenesis begins with endothelial dysfunction which attracts and promotes monocyte adhesion to the endothelium. Monocytes transmigrate through the endothelium in the sub-endothelial space, differentiate into macrophages, phagocytose oxidized low-density lipoprotein (oxLDL) and become foam cells. Simultaneously, smooth muscle cells (SMCs) migrate in the sub-endothelial space, phagocytose the oxLDL and transform also into foam cells. Atheromatous plaque is formed with a fibrous cap. Fibrous plaques are often vulnerable to rupture causing thrombosis and stenosis [4-6].

We herein review the contribution of NHE-1 in atherogenesis (namely its effect on endothelial cells, monocytes, SMCs and platelets). We also consider the relationship between NHE-1 and vascular risk factors such as obesity, diabetes mellitus, hypertension, dyslipidemia and inflammation. Even though clinical trials with

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Article highlights.

- The sodium/hydrogen exchanger-1 (NHE-1) is a ubiquitous plasma membrane protein whose main role is to maintain intracellular pH and volume.
- NHE-1 plays a role in monocyte differentiation, adhesion, migration, CD36 expression and phagocytosis of oxidized low-density lipoprotein.
- NHE-1 can influence smooth muscle cell and platelet function.
- NHE-1 activity is increased in red blood cells obtained from obese humans.
- High concentrations of glucose or insulin can activate NHE-1 suggesting that NHE-1 activity might be increased in diabetes.
- Whether NHE-1 inhibition can be used to treat hypertension remains to be established.
- Cholesterol can influence NHE-1 activity.
- NHE-1 inhibition can improve inflammatory markers.
- Several commonly used drugs (e.g., enalapril, nifedipine, lisinopril and valsartan) seem to modulate NHE-1 activity.
- Even though clinical trials with NHE-1 inhibitors reported discouraging results in terms of vascular events, these drugs merit further investigation.

This box summarizes key points contained in the article.

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certain NHE-1 inhibitors had discouraging results in the treatment of ischemic cardiac events, NHE-1 cannot be excluded as a potential future therapeutic target [7-10]. It has to be noted that there is no known clinical trial for the evaluation of NHE1 inhibition for the prevention or treatment of atherosclerosis.

2. Effect of NHE-1 on cellular level

2.1 Endothelial cells

Endothelial cells form the inner lining of the vasculature [4]. Circulating leukocytes normally do not adhere to the endothelium, mainly due to local production of NO, prostacyclin and CD39 [11]. Dysfunctional endothelium promotes monocyte rolling, adherence and transmigration into the sub-endothelial space through increased expression of adhesion molecules (e.g., intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule, P- and E-selectins and integrins) and chemokines (e.g., monocyte chemoattractant protein-1, MCP-1) [4].

Endothelial function is influenced by NHE-1 activity. Inhibition of NHE-1 preserves endothelial-dependent vasorelaxation after ischemia and prevents endothelial dysfunction. When reperfused after ischemia, isolated rat hearts showed impaired endothelial-dependent acetylcholine-induced vasorelaxation, which was prevented with SM-20550 (a NHE-1 inhibitor) administration pre- and post-ischemia [12]. Similar findings have been reported in animal models with other NHE-1 inhibitors such as cariporide [13-15],

benzamide, N-(aminoiminomethyl)-4-[4-(2-furanylcrbonyl)-1-piperazinyl]-3-(methylsulfonyl), methanesulfonate] (BIIB-513) [16] and eniporide [17]. In the last study, eniporide preserved endothelial function (estimated by means of acetylcholine-induced vasorelaxation) after 90 min of ischemia in dog hearts. NHE inhibition in *ex vivo* cultures protected endothelial cells against cell injury and death [12]; thus, it could prove beneficial in states of endothelial dysfunction.

In contrast with these findings, another study reported that *in vivo* inhibition of NHE in rat basilar artery prevented the acetylcholine- and bradykinin-induced vasodilatation but did not affect the baseline artery diameter. Hence, intracellular alkalinization mediated by NHE activation could enable NO production in basilar artery endothelium [18]. Yet, the reason for this paradoxical finding is not obvious. It could reflect response variability in different vascular beds, which could be attributed to variations in receptor distribution according to the anatomical location of arteries in experimental models [19]. Another possible explanation is the pre-existing ischemia in some experiments which predispose to a different role of NHE. Ischemia-reperfusion promotes the local production of free radicals (reactive oxygen species (ROS)) and an interaction between NHE1-related signaling and ROS has been reported [20,21]. Heat shock protein 70, a molecule related to oxidative stress, seems to be attached to the regulatory domain of NHE; however, the role of this association is not yet clear [22].

Endothelial dysfunction in subjects with diabetes might also be related to NHE [23]. High glucose concentrations activate NHE, thereby, inhibiting NO production via a dissociation of eNOS from heat shock protein 90 in HUVECs [23]. Cariporide (a specific inhibitor of NHE-1) [24] and siRNA for NHE reversed the above-mentioned results [23]. Moreover, administration of cariporide in diabetic animal models has been associated with coronary endothelium protection [25].

The endothelium produces a number of vasoactive substances (e.g., NO, prostacyclin) and NHE-1 could play a role in this process [26]. However, inhibition of NHE does not affect basal production of platelet derived growth factor (PDGF) and monocyte adhesion to endothelial cells but does inhibit thrombin-induced PDGF production by human aortic endothelial cells [27].

The interaction between endothelial cells and leukocytes (mainly neutrophils) is influenced by NHE-1. Cariporide inhibits leukocyte rolling, adhesion and transmigration in states such as post-ischemia reperfusion, where these leukocyte properties are enhanced. However, it has no effect in normal states where leukocyte rolling, adhesion and transmigration are not increased [28]. Thus, pharmacological inhibition is beneficial only when it is highly selective in disease states where undesirable effect occurs (e.g., excessive leukocyte transmigration). The aforementioned interaction between endothelium and leukocytes is regulated by the expression of adhesion molecules such as ICAM-1, P- and E-selectins as

well as MCP-1. Inhibition of NHE-1 counteracts the high glucose-induced increase in ICAM-1 expression in endothelial cells and the subsequent monocyte adhesion to endothelial cells [29]. P-selectin expression is also inhibited by cariporide in states of increased expression [28]. Similar findings have been reported for E-selectin and MCP-1 when NHE-1 is inhibited by amiloride [30]. Other blood cells such as platelets are also influenced by NHE-1 inhibition and show decreased adhesion to endothelium following cariporide administration [31].

Furthermore, amiloride derivatives, mainly 5-(*N*,*N*-hexamethylene)-amiloride, cause apoptosis of endothelial cells and upregulation of COX-2 (which is involved in the inflammatory response of endothelial cells) and several genes in endoplasmic reticulum [32].

2.2 Monocytes

Monocytes play a key role in atherogenesis following their recruitment and attachment to the activated endothelial cells through increased expression of leukocyte adhesion molecules [5,33,34]. Subsequently, monocytes migrate into the intima media under the influence of chemokines, differentiate to macrophages, proliferate and form foam cells [4,5].

NHE plays a significant role in the differentiation process of monocytes. Studies with HL60 promyelocytic leukemia cell lines which differentiate into monocytes/macrophages have shown that cell differentiation is preceded by a NHE mRNA and protein synthesis increase [35]. Similarly, in stem cells which were treated with dimethyl sulfoxide in order to differentiate to cardiomyocytes, NHE-1 inhibition prevented cell differentiation [36] and increased expression of NHE-1 from an adenoviral vector promoted cardiomyocyte cell differentiation [37]. Moreover, several monocyte properties involved in atherogenesis seem to be mediated by NHE-1. These have all been examined following enhancement by a hormone. NHE-1 inhibition has been shown to prevent this enhancement. Leptin, adrenaline, endothelin-1 and high glucose concentrations increased monocyte adhesion, migration, CD36 expression and phagocytosis of oxLDL via NHE-1-dependent mechanisms [21,38-42]. Furthermore, inhibition of NHE-1 activity with cariporide prevents the lysophosphatidylcholine (LPC)-induced adhesion of monocytes to bovine aortic SMCs [43]. It is worth noting that cariporide has no effect on monocyte adhesion to SMCs in the absence of LPC [43]. This finding is similar to the one reported for endothelial cells where NHE-1 inhibition seems to have a beneficial effect only when there is activation of a process but not under basal conditions.

2.3 SMCs

SMCs influence plaque stability [44]. SMCs under the influence of growth factors migrate into the intima [45] and change their phenotype from contractile to secretory. These secretory SMCs proliferate and produce collagen and several inflammatory mediators enhancing the atherogenic process [4,46].

NHE-1 has a regulatory role in the maintenance of intracellular pH (pHi) in SMCs [47]. The physiologic function of the vascular wall is influenced by SMC pHi; acute intracellular acidification causes contraction [48,49]. Activation of NHE-1 in SMCs has been observed following *in vitro* addition of noradrenaline and phenylephrine suggesting an involvement of pHi in SMC contraction [50]. Furthermore, SMC growth is modulated by pHi [51]. NHE is localized in the plasma membrane but is also found at the nuclear membrane [52]; thus, it could play a role in the function of nucleus in SMCs.

Studies report that NHE-1 mediates several aspects of SMC function such as proliferation, adhesion, migration, molecule expression and apoptosis. Inhibition of NHE-1 with a selective inhibitor, sabiporide, results in the arrest of cell cycle progression at the G_0/G_1 phase and inhibits pulmonary SMC proliferation. Under basal conditions, SMCs are in non-proliferative state while in atherosclerosis they proliferate. Arresting the cell cycle of SMCs could prevent the progression of atherogenesis. In the same cell type, sabiporide inhibits cell migration in a dose-dependent manner [53] indicating that NHE-1 is involved in SMC migration. The level of NHE-1 message varies in response to the state of SMC proliferation. NHE-1 mRNA are increased in SMCs during exponential growth, but decrease at confluency. SMCs deficient in NHE-1 have reduced growth. Injury to the vascular wall increases NHE-1 mRNA levels as does pressure overload of hearts. These results suggest that NHE-1 is involved in vascular SMC proliferation [54].

NHE-1 seems to also be involved in LPC-induced increase in ICAM-1 expression by SMCs and monocyte adhesion to them [43] as cariporide has been shown to inhibit ICAM-1 expression on SMCs in a dose-dependent manner [43]. However, ICAM-1 expression on SMCs is not affected by cariporide in the absence of LPC [43].

NHE-1 inhibition causes apoptosis of SMCs [55] possibly due to cellular acidification, a finding that has been shown both in peripheral blood monocytes and leukemic cells [56,57].

There is evidence supporting that NHE-1 could contribute to the differences in vascular risk between males and females. A study reports that low physiologic concentrations of 17-β-estradiol activate NHE-1 and increase pHi in rat SMCs. However, high levels of 17-β-estradiol did not affect pHi which could contribute to the different risk for atherosclerosis in men and postmenopausal women compared with premenopausal women [50]. The activation of NHE-1 is possibly through a nongenomic mechanism in these cells.

2.4 Platelets

Platelets are activated after contact with sub-endothelial layers which contain collagen and extracellular matrix proteins leading to platelet aggregation. Another important role of platelets is the production of inflammatory proteins, chemokines and proliferative factors [4,58,59]. A variety of agonists stimulate NHE activity in platelets including thrombin, arachidonic

acid, platelet activating factor, angiotensin II, endothelin and phorbol esters [60]. Several novel NHE-1 inhibitors have been reported to prevent platelet swelling [61]. An increase in median platelet volume (MPV) is an early phase of platelet activation [62]. Whether NHE-1 inhibitors block agonist-induced increase in MPV remains to be established. Furthermore, it is not known if the platelet swelling that is influenced by NHE-1 inhibitors reflects the same process as an agonist-induced increase in MPV.

Following activation, platelets release microvesicles with pro-coagulant activity [63]. Inhibition of NHE-1 with 5-(N-ethyl-N-isopropyl) amiloride (EIPA) prevents collagen- and phorbol ester-evoked vesiculation [64]. Stimulation of porcine platelets by thrombin not only results in NHE-1 activation but also in secretion of serotonin [65]. Inhibition of NHE-1 prevents thrombin-induced secretion of serotonin [65,66]. Serotonin is an activator of platelets, a vasoconstrictor and acts as a proliferator of SMCs. Therefore, this bioamine may play a role in atherogenesis and thrombogenesis [67,68].

After platelet activation, phosphatidyl serine (PS) on the plasma membrane is exposed and initiates the coagulation cascade. NHE-1 seems to be involved in this process. Inhibition of NHE-1 with EIPA results in decreased exposure of PS in human platelets [69]. A similar finding has been observed when PS expression was stimulated by arginine vasopressin [70].

Another inhibitor of NHE-1 (KR-32560) prevents collagen-induced platelet aggregation possibly through inhibition of cytoplasmic Ca^{2+} mobilization and arachidonic acid liberation [71]. Similarly, inhibition of NHE-1 with EIPA prevents desmopressin-evoked platelet pro-coagulant activity [72]. Besides platelet aggregation, inhibition of NHE-1 has been shown to decrease platelet–leukocyte aggregation [73].

All these findings indicate that NHE-1 is involved in platelet activation, serotonin release, platelet aggregation and interaction with other cell types. Furthermore, inhibition of NHE-1 seems to favorably influence platelet function suggesting that NHE-1 inhibitors could contribute to atherogenesis prevention and treatment. It appears that the presence of sodium ion in the medium is required for platelet aggregation [60].

3. NHE-1 and vascular risk factors

3.1 Obesity

NHE-1 activity is increased in red blood cells (RBCs) obtained from obese humans and correlates with body mass index [74]. Furthermore, NHE-1 mediates the atherosclerotic properties of monocytes (adhesion, migration, CD36 expression and oxLDL phagocytosis) from obese and lean subjects [39,41,42,75]. A possible link between obesity and accelerated atherogenesis could be the increased NHE-1 activity in this state. This may be attributed to the several hormonal changes associated with obesity, such as increased insulin and leptin plasma levels [76] or increased

sympathetic activity [77-79]. In this regard, efficacy of several hormones in stimulation of NHE-1 activity has been examined, including insulin, leptin and adrenaline. High concentrations of insulin increased NHE-1 activity in human RBCs [80] obtained from lean healthy subjects but not from obese subjects [81]. However, insulin activated NHE-1 in human monocytes obtained from obese subjects [82]. Leptin increased NHE-1 activity in RBCs obtained from lean and obese humans. However, the increase was greater in the lean group compared with the obese group [83]. This difference could potentially be explained by the existence of leptin resistance in RBCs obtained from obese humans. Finally, adrenaline activated NHE-1 in human RBCs and the response was greater in the RBCs obtained from the obese compared with the lean subjects [84].

Though the aforementioned hormones exhibit a different NHE-1 response in obese subjects compared with the lean, the effect varied with adrenaline having a greater stimulatory effect and insulin and leptin having less or no effect on NHE-1 activity. In the obese, insulin, leptin and adrenaline levels may be increased [76,79] which could contribute to altered NHE-1 activity. Interestingly, there are no data whether obese subjects have increased NHE-1 mRNA or protein expression.

3.2 Diabetes

Diabetes is a risk factor for atherosclerosis. High concentrations of glucose activate NHE-1 [40,85] in a time-dependent manner and this effect was inhibited by cariporide [23]. Furthermore, high glucose concentrations induce impairment of endothelium-dependent vasorelaxation in rat aortic rings. This effect is concentration-dependent [86] and attenuates after administration of cariporide [86] in a dose-dependent manner [87]. Supporting this finding, cariporide prevented endothelial dysfunction in diabetic rats [23]. Furthermore, chronic treatment with cariporide improved endothelial function in obese mice [88]. This evidence suggests that inhibition of NHE-1 could be beneficial in diabetes.

In insulin-resistant animal models, cariporide reduced fasting insulin levels and insulin response to meal tolerance tests indicating improved insulin sensitivity [88]. Furthermore, insulin sensitivity was improved in patients with type 2 diabetes after oral calcium supplementation and was accompanied by decreased activity of NHE-1 in platelets [89]. However, the effect of NHE-1 on insulin sensitivity needs to be investigated.

Improvement of hyperinsulinemia with troglitazone for 4 weeks reduces NHE-1 activity in platelets obtained from fructose-fed borderline hypertensive rats. Moreover, a positive correlation between plasma insulin concentrations and NHE-1 activity (r = 0.27, p = 0.01) was reported in this study [90].

Insulin inhibited NHE-1 activity in mouse mesenteric artery smooth muscle and endothelial cells through the involvement of H$_2$O$_2$ [91]. In this study, the insulin

concentration used (2.5 – 800 nmol/l) was about 10 – 2500 times more than the insulin concentrations encountered in insulin-resistant or diabetic humans (about 0.3 nmol/l). Other studies showed that insulin activates NHE-1 in human monocytes [75] and RBCs [81]. In contrast, others have shown [92,93] that NHE activity is decreased in papillary muscles from the hearts of diabetic rats. This may be due to diabetes-related alterations in Ca/calmodulin protein kinase II. The effect of insulin on NHE-1 activity has raised a controversy whether it is stimulatory or inhibitory. Vaughan-Jones and Swietach in a recent discussion of the matter have concluded that possibly there is a balance between stimulatory and inhibitory actions with a modest overall stimulatory effect [94].

In conclusion, there is evidence that high concentrations of glucose or insulin could activate NHE-1 suggesting that NHE-1 activity might be increased in diabetes. Attenuation of hyperinsulinemia reduces NHE-1 activity. NHE-1 inhibition is also associated with improved insulin sensitivity and prevents endothelial dysfunction. Thus, NHE-1 inhibition in diabetes might have a dual benefit: prevention/treatment of atherogenesis and improved insulin sensitivity. But, this suggestion needs to be proven. It should be noted that in the diabetic myocardium, NHE-1 activity is decreased though it is not clear whether this is a secondary effect.

3.3 Hypertension

Animal and *ex vivo* studies with human peripheral blood cells have documented an increased activity of NHE-1 in primary hypertension [95]. However, genetic linkage studies have excluded the NHE-1 locus from primary hypertension candidate genes [96,97]. Nevertheless, elevated NHE-1 activity has been persistently demonstrated in humans with hypertension and in animals models [98] and it has been suggested that regulation of NHE-1 activity may be affected in hypertension [98]. Alternatively, several newly discovered isoforms of NHE may be responsible for the elevated activity [99].

Inhibition of NHE-1 in spontaneous hypertensive rats with EIPA or amiloride prevented acetylcholine-induced pulmonary artery contraction. However, EIPA or amiloride did not have any effect on the wild-type rats [100]. This finding indicates a different response of hypertensive animals to NHE-1 inhibitors possibly due to altered activity of NHE-1 in these models. So, NHE-1 activity might contribute to the development of hypertension.

NHE overactivity is reported in human lymphocytes obtained from hypertensive patients with obesity or type 2 diabetes [101]. Hyperinsulinemia attenuation following treatment with troglitazone for 4 weeks reduces NHE-1 activity in platelets obtained from fructose-fed borderline hypertensive rats [90].

NHE-1 seems to be associated with hypertension but it still needs to be proven whether NHE-1 contributes to hypertension development or whether NHE-1 inhibition could be used in treatment of hypertension. The discovery

of new NHE isoforms that may be linked with hypertension is exciting and also requires further investigation. Sodium lithium countertransport is elevated in hypertension. A report [102] suggested that a splice variant of NHE1 is responsible for this. However, further studies are necessary to determine the role this splice variant plays in hypertension.

3.4 Dyslipidemia

Several studies have reported that NHE-1 activity is influenced by dyslipidemia. There were reports that in hypercholesterolemic ApoE$^{-/-}$ mice, ischemia and reperfusion caused a smaller infarct size compared with the healthy wild-type mice. This paradoxical finding was explained by the downregulation of NHE-1 protein in cardiomyocytes. Furthermore, histopathology showed no coronary atherosclerosis in these hearts [103]. In accordance with these findings, thrombin-induced NHE-1 activation was reduced in platelets obtained from hereditary hypertensive hypercholesterolemic rats compared with healthy controls [104]. However, there was no difference between these animal models and the healthy controls in basal platelet pHi [104].

It is obvious that high cholesterol levels can alter NHE-1 activity and several studies have proposed underlying mechanisms. LDL inhibits activation of NHE-1 in human platelets in a dose-dependent manner [66], probably through activation of p38^{MAP} kinase [105]. Normally, activation of platelets is accompanied by an initial decrease of pHi, which is counteracted by NHE-1 activity [60]. This mechanism is less pronounced in platelets obtained from humans with familial hypercholesterolemia compared with those obtained from healthy controls [66]. Furthermore, lowering circulating LDL-cholesterol (LDL-C) levels in familial hypercholesterolemia (achieved by LDL apheresis) increased NHE activity [66]. The effect of LDL on NHE-1 resembles the action of NHE-1 inhibitor HOE 694 [66].

High-density lipoprotein (HDL) exerts an opposite effect to LDL on NHE-1 activity [106]. This is an interesting finding as HDL commonly functions in opposition to LDL [107]. HDL$_3$ enhanced NHE-1 activity after stimulation with acidification even though it had no effect under basal conditions [106]. The proposed mechanism of HDL$_3$ enhancing effect on NHE-1 is via binding to glycoproteins IIb/IIIa and activation of PKC and phosphatidylcholine-specific phospholipase C [106].

As mentioned earlier, lowering LDL-C enhances NHE-1 activity. However, improvement of hypertriglyceridemia with bezafibrate for 4 weeks did not affect NHE-1 activity [90].

In conclusion, NHE-1 activity seems to be reduced in hypercholesterolemic animal models probably due to increased LDL-C, which reduces NHE-1 activity, or decreased HDL, which enhances NHE-1 activity. However, the clinical relevance of this finding is not yet clear. Biochemically, it has been shown that cholesterol affects NHE-1 activity. Cholesterol

depletion stimulated NHE-1 activity and was associated with relocation outside microdomains [108].

3.5 Smoking

Surprisingly, we could not find any relevant literature linking smoking and NHE-1 activity. However, as NHE 1 activity is influenced by free radicals [109] and cigarette smoking is strongly associated with free radical production [110], an association between NHE 1 activation and smoking cannot be excluded.

3.6 Inflammation

Some emerging risk factors for atherosclerosis have been demonstrated to improve with NHE-1 inhibition. In animal models with heart failure after myocardial infarction (MI), cariporide administration decreased the levels of CRP (C-reactive protein) compared with the placebo group [111]. Inhibition of NHE by either removing extra-platelet Na$^+$ or adding an amiloride derivative also decreased arachidonic acid release from platelets [112].

More direct anti-inflammatory effects have also been shown. The anti-inflammatory effect of NHE-1 has been shown *in vivo* and *in vitro* in an animal model; NHE-1 inhibition decreased prostaglandin E2 production through the inhibition of arachidonic acid release and increased the levels of COX-2 protein [113]. In experimental colitis, NHE-1 inhibition resulted in reduction of certain inflammatory markers (myeloperoxidase and IL-1β) [114]. Further, in endotoxin-exposed endothelial cells, NHE-1 inhibition decreased the inflammatory response of the cells by suppression of IL-8 production, inhibitory-κB and NF-κB DNA binding [30]. The anti-inflammatory effects of NHE-1 inhibition and improving CRP levels can result in lower risk of vascular events [115,116]. We showed that ROS trigger increased NHE-1 gene and protein expression [117]. Given the role of ROS in atherosclerosis [118,119], this action may also be relevant.

4. Clinical trials and potential therapeutic applications

The use of several NHE-1 inhibitors in cardiovascular disease has been investigated during the past decade. Animal post-MI studies have shown that NHE-1 inhibition could limit reperfusion injury in the heart [120] (reviewed in [121]) and improve contractile function [122]. Cariporide in combination with metoprolol decreased MI size in animal models [123]. Furthermore, NHE-1 also has a prohypertrophic effect and its inhibition can reduce or prevent heart hypertrophy [121].

A number of clinical studies have examined the efficacy of several NHE-1 inhibitors in the treatment of cardiovascular disease. In the Guard During Ischemia Against Necrosis (GUARDIAN) trial, cariporide was evaluated in patients at risk of myocardial necrosis [124]. Patients with non-ST elevation acute coronary syndrome were enrolled and randomized to cariporide 20, 80 or 120 mg intravenously 3 times per day or placebo for 2 – 7 days. There was no benefit in the ventricular wall motion score index or in the number of abnormal chords [9]. Analysis of a patients cohort undergoing coronary artery bypass graft surgery (CABG) showed that only the highest dose of cariporide (120 mg) decreased all-cause mortality and MI in the first 36 h postoperatively (p = 0.005); the beneficial effect remained for 6 months (p = 0.033) [125]. In this cohort, patients were randomized to placebo (n = 743) or cariporide 20 mg (n = 736), 80 mg (n = 705) or 120 mg (n = 734). A 1 h intravenous infusion was initiated shortly before surgery and administered every 8 h for 2 – 7 days. The lower doses of cariporide (i.e., 20 or 80 mg) showed no benefit versus placebo [125]. Cariporide seemed to be well tolerated with mild and transient side effects [126].

The EXPEDITION trial (Na$^+$/H$^+$ Exchange inhibition to Prevent coronary Events in acute cardiac condition) aimed to investigate the effect of cariporide (180 mg in a 1 h pre-CABG loading intravenous dose, then 40 mg/h over 24 h and 20 mg/h over the subsequent 24 h) or placebo on death and MI prevention in patients undergoing CABG (n = 5761). There was a decrease in MIs (18.9% placebo group vs 14.4% cariporide group, p < 0.001) but an increase in mortality (1.5% placebo group vs 2.2% cariporide group, p = 0.02) mainly due to cerebrovascular events [7]. There was an excess of ischemic strokes in this study in the cariporide group (124/2870 vs 67/2839; p < 0.0001 calculated by us using the chi-square test with Yates correction). It is of interest that an experimental model has shown that inhibition of NHE-1 activity (using 5-N,N-hexamethyleneamiloride or FR183998) prevented the acetylcholine-induced vasodilatation of basilar artery [18].

Another study (n = 824) with an inhibitor of NHE-1, zoniporide, showed no benefit in reducing composite cardiovascular end point in patients with multiple risk factors for coronary disease undergoing non-cardiac surgery. In this study, all four groups (placebo, zoniporide 3-, 6-, or 12-mg/kg/day) received a loading dose 1 h before the operation and postoperatively an intravenous maintenance dose for a period of 24 h up to 7 days [127].

In the Evaluation of the Safety and Cardioprotective effects of Eniporide in Acute Myocardial Infarction (ESCAMI) trial, eniporide was infused 10 min before the procedure in patients with ST elevation MI undergoing thrombolytic therapy or angioplasty surgery. Patients were randomized in stage 1 (n = 430) to 50, 100, 150 or 200 mg eniporide or placebo and in stage 2 (n = 959) to 100 and 150 mg eniporide or placebo. Eniporide versus placebo did not limit MI size or improve clinical outcome [10]. More recently, the pharmacokinetics of eniporide and its metabolite (EMD 112 843) were investigated in healthy subjects and in patients undergoing myocardial reperfusion therapy [128].

The majority of the aforementioned trials have not shown a clinical benefit for NHE-1 inhibitors in patients with

coronary heart disease. A benefit has only been shown in patients undergoing CABG receiving 120 mg dose of cariporide 1 h before CABG and postoperatively every 8 h for 2 – 7 days (GUARDIAN trial) [124]. However, overall mortality was significantly increased (p = 0.02) in a later study that also enrolled patients undergoing CABG (EXPEDITION trial) [7]. The opposing findings of these trials could be explained by the differences in the dose administration protocol. Furthermore, the number of patients involved in the studies differed considerably (n = 1477 at 120 mg dosage of cariporide in the positive trial (GUARDIAN) and n = 5761 in the negative trial (EXPEDITION)).

Newer agents are being investigated as potent inhibitors of NHE-1 with better cardioprotective effects in animal models than the older inhibitors [61,129-133]. Furthermore, an orally administered agent (SMP-300) has also been used in experimental angina and shown better results in infarct size compared with propranolol and nifedipine [134]. Future studies may establish if NHE-1 inhibitors are of clinical benefit. It should be highlighted that all the above-mentioned drugs do not solely inhibit NHE-1 but also other isoforms of NHE with different though lower efficacy [24]. So, it is possible that some of the observed effects in these clinical trials could be caused by inhibition of other NHE isoforms. Also, the presence or absence of bicarbonate in the incubation medium in experiments may influence the results with bicarbonate dependent transport being more of a contributory factor when substrate is available [135].

5. Modulation of NHE-1 activity by clinically used compounds

Several widely used drugs seem to modulate NHE-1 activity. In hypertensive animal models, the administration of enalapril (20 mg/kg/day), nifedipine (10 mg/kg/day) or losartan (40 mg/kg/day) resulted in normalized NHE-1 activity [136]. In young adults with mild hypertension, lisinopril was used for 12 weeks and decreased NHE activity in RBCs [137]. Another ACE inhibitor, quinapril decreased NHE activity in lymphocytes from hypertensive patients after 6 months of treatment [138]. Similarly, enalapril treatment of spontaneous hypertensive rats normalized NHE activity in myocytes [139]. Ramipril and valsartan decreased NHE-1 activity in post-MI animal models mainly due to inhibition of mRNA and protein levels [140]. Long-term (7 days) cilaxapril treatment of hypertensive

animal models prevented the increase in NHE-1 expression [141]. Different results were reported with captopril which increased NHE activity in RBCs from hypertensive patients [142].

In other studies, skeletal muscle NHE was activated by isoprenaline, which stimulates β_2-adrenoceptors, *in vivo*. This effect was attenuated by nifedipine [143]. Conversely, sildenafil was shown to inhibit cardiac NHE-1 activity and this effect was associated with protecting the myocardium against post-MI remodeling and dysfunction [144].

6. Expert opinion

NHE-1 is involved in the function of all cell types (endothelial cells, monocytes, SMCs and platelets) associated with atherogenesis and with several vascular risk factors (obesity, diabetes, hypertension, dyslipidemia and inflammation). There is evidence that NHE-1 contributes to atherogenesis but it does require further investigation. It is of interest that commonly used drugs (e.g., anti-hypertensive) modulate NHE-1 activity but the clinical relevance of such an effect remains unclear. Furthermore, in many experimental settings, inhibition of NHE-1 has an effect only in states of increased NHE-1 activity. In conclusion, NHE-1 inhibition could contribute to atherogenesis prevention and treatment as long as safe NHE-1 inhibitors are found. Yet, the main limitation of this class of drugs is its lack of specificity.

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Declaration of interest

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